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Quantitative Light-Induced Fluorescence to Measure Enamel Remineralization in vitro

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Key Words
Fluoride · Quantitative light-induced fluorescence · Remineralization · Surface microhardness

Abstract
The aim of this study was to compare the ability of quantitative light-induced fluorescence (QLF) and surface microhardness (SMH) to measure the remineralization of enamel subsurface lesions, using a pH-cycling model including treatment with 0-ppm, 550-ppm or 1,100-ppm sodium fluoride (NaF) dentifrices. ***Methods:*** Subsurface lesions were created in human enamel specimens (n = 36) and exposed to a remineralization pH-cycling model for 14 days. The pH-cycling model was performed in an automated system where specimens were subjected to a demineralizing solution for 20 min and treatment for 1 min and were then remineralized for 7 h 39 min, 3 times daily. The treatments consisted of 3 NaF, silica-containing dentifrices (0 ppm F; 550 ppm F; 1,100 ppm F). The outcome variables were: change from baseline in surface hardness and percentage change from baseline in fluorescence. An ANCOVA explored differences between different treatment groups (at the $p < 0.05$ level). Associations between QLF and SMH were evaluated using Spearman's correlation coefficient. ***Results:*** The percentage SMH changes were 14.9 ± 2.1%, 56.6 ± 9.6% and 103.9 ± 14.6% for the 0-, 550- and 1,100-ppm F dentifrices, respectively. The percentage fluorescence changes were 15.6 ± 7.1%, 59.8 ± 11.9% and 85 ± 13.2%, respectively. The differences between all pairwise comparisons were statistically significant for both methods ($p = 0.001$). QLF correlated with SMH ($r = 0.67$). ***Conclusions:*** Both the SMH and QLF methods demonstrated a significant F dose response for toothpaste in this in vitro remineralization model, and both methods were able to distinguish treatments with different F levels.

There has been an overall decline in the prevalence, extent and severity of dental caries in children and young adults around the world, particularly in developed countries [Marthaler, 1990]. The evidence supporting the use of fluorides to prevent dental caries by reducing demineralization and enhancing remineralization is unequivocal and has contributed significantly to this decline [Bratthall et al., 1996; Marinho et al., 2003].

In vitro de- and remineralization models of enamel caries lesions have been developed to assess the anticaries potential of fluoride and other factors influencing caries lesion progression and repair [White, 1995]. The slower rate of progression of caries today, and the development of more sensitive methods of detection, have prompted caries researchers to examine smaller, initial mineral changes in in vitro and in vivo studies [Nyvad, 2004]. Re-

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cently, researchers have shown an increased interest in non-destructive methods such as quantitative light-induced fluorescence (QLF) for the quantitative assessment and longitudinal monitoring of mineral changes in the early stages of caries [Pretty, 2006]. A consensus meeting [Pitts and Stamm, 2004] and a recent review [Ellwood et al., 2012] both concluded that QLF may help to reduce subject numbers and trial duration when assessing the efficacy of new anticaries treatments.

QLF is a system based on the measurement of fluorescence loss following enamel demineralization [Pretty, 2006]. This method has been employed in pH-cycling experiments [Hafström-Björkman et al., 1992] and in monitoring in vivo remineralization of white spot lesions [Al-Khateeb et al., 1998; Tranaeus et al., 2001; Feng et al., 2007; Karlsson, 2010]. The purpose of this study was to determine the ability of QLF to detect differences in remineralization of artificial caries lesions in a pH-cycling model using 3 types of silica-based NaF toothpastes (0 ppm, 550 ppm and 1,100 ppm) and compare changes to those measured using surface microhardness (SMH).

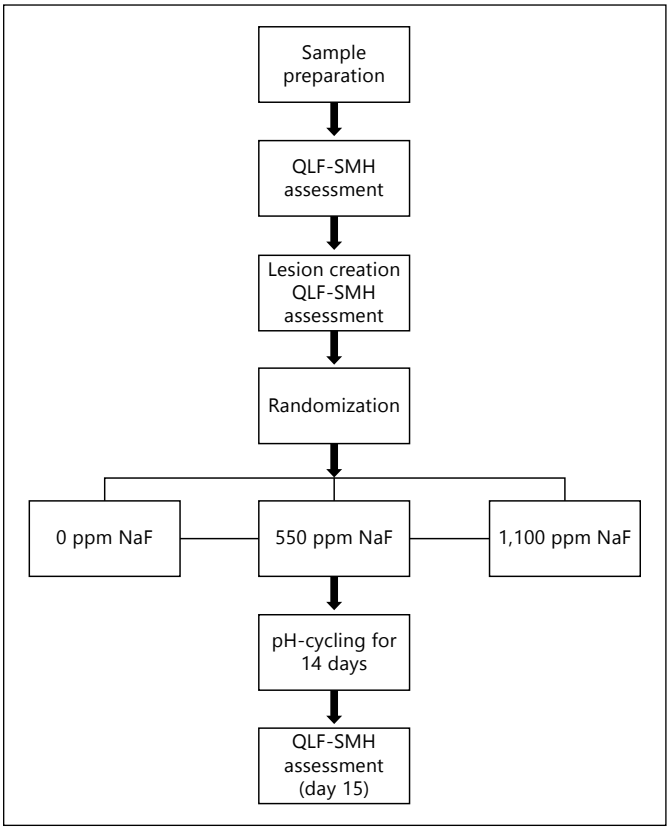

Fig. 1. Flowchart of the study design.

Methods

Extracted human molar teeth were collected from dental surgeries located in the state of New Jersey, USA. Informed consent was obtained from the patients prior to the extractions. The teeth were stored in 10% (v/v) neutral phosphate-buffered formalin for a minimum of 2 weeks. The selected teeth did not have any developmental defects, cracks or white spot lesions. Figure 1 illustrates the study design employed.

Enamel Specimen Preparation

Human extracted teeth were cut into 3 or 4 parts with a water-cooled diamond saw. Thirty-six slices were obtained, ground flat and polished using 3 retaining rings and a 15-μm diamond polishing disc (Buehler, Lake Bluff, Ill., USA). QLF images were taken before the creation of the lesions and used as a reference area for the analysis. Specimens were selected based on their sound surface Knoop hardness number (KHN). The mean KHN of the enamel blocks selected for this study was 343.1 ± 30 kg/mm^2.

Double-sided adhesive strips were attached to both sides of the sample so that a central area of approximately 6 mm^2 was exposed. During the evaluations, the specimens were kept in 100% relative humidity at 4°C. The specimens were air dried for 5 s with compressed air fixed at 10 cm from the teeth before QLF and SMH assessment.

Lesion Preparation

Caries-like lesions were formed in the exposed enamel after immersion for 32 h [Queiroz et al., 2008] at 28°C in a pH 4.6 solution of 0.1 mol/l acetic acid containing 800 mg/l dissolved hydroxyapatite and 5 g/l of Carbopol 981. SMH assessment and QLF measurements were carried out [White, 1995], and samples were randomly assigned to 3 groups based on the value of the KHN after lesion creation. Lesions were stratified and assigned to 3 treatment groups (n = 12 per group). The lesion average baseline values were 93.99 ± 27.87, 92.48 ± 25.95 and 92.1 ± 20.83 for SMH and the average fluorescence loss values were 28.6 ± 3.3, 20.2 ± 4.4 and 24.3 ± 4.5 for the 0-, 550- and 1,100-ppm F treatments, respectively.

Table 1. pH-cycling regime

Treatment	Time		
Rinse, s	6		
Demineralization, min	20		
Rinse, s	6	↑	Conduct 3 times/day; repeat for 13 additional days
Treatment, min	1		
Rinse, s	6		
Remineralization, h:min	7:39		

pH Cycling

The pH-cycling model (table 1) was performed by a custom-made robot system for 14 days. The samples were consecutively subjected to a demineralizing solution for 20 min, then to 1 of 3 treatments under agitation for 1 min and to a remineralizing solution for 7 h 39 min, 3 times a day. After each demineralization,

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treatment and remineralization period, the samples were rinsed with deionized water for 6 s. Demineralization and remineralization solutions were prepared according to the compositions shown in table 2 [Stookey et al., 2011].

The treatments consisted of 3 silica-based NaF dentifrices (0-ppm F: group 1; 550 ppm F: group 2; 1,100 ppm F: group 3). The specimens were treated for a period of 1 min with dentifrice and water slurries (1 part dentifrice, 3 parts deionized water) to represent the level of dilution that occurs during routine use of toothpaste products. All the solutions were changed daily. After 14 days of cycling, QLF measurements and SMH assessments were performed.

QLF Customized System

After treatment, the tape was removed from the specimens, which were then washed and dried for 5 s before QLF images were captured. A customized QLF setup was employed, consisting of blue light-emitting diodes in a ring illuminator emitting light with the peak source wavelength at 405 nm and a 3-charged coupled device colour camera installed with a long-pass yellow filter (495 nm; Schott, Stafford, UK). A 35-mm focal-length imaging lens was used to capture a field of view of approximately 330 by 450 mm. The images were captured in a lightproof enclosure using custom software. Video repositioning software was used to ensure that images were automatically captured when the correlation was higher than 0.90, to ensure consistent capture areas. The difference between the green pixel values in the reference area and those in the lesion area was divided by the reference area (green pixel values) at baseline and after treatment. This difference was expressed as a change in fluorescence.

Surface Microhardness

SMH measurements were taken with a Knoop diamond at a constant load of 50 g*F* applied for 15 s using a Buehler Micromet 5105 tester (Buehler). The teeth were placed flat on the translation stage and fixed at a reproducible position within the microindentor. Four indentations were made on each specimen during each measurement time point at spacing of approximately 100 μm apart. The KHN were calculated and averaged at each time point. The outcome of SMH change was calculated based upon the differences between the KHN values at baseline (lesion area) and after treatment.

Statistical Analysis

Data were analysed using SPSS-PC (version 19). The variables analysed were: change from baseline in surface hardness and change from baseline in fluorescence.

Differences between different treatment groups were tested for significance at the $p < 0.05$ level by an ANCOVA, using the baseline measurement as a covariate. All 3 pairwise comparisons were assessed using the Bonferroni correction for multiple comparisons. The strength of the association between QLF and SMH was evaluated with the Spearman correlation coefficient.

Table 2. Composition and elements used in the pH-cycling model

Samples	Human enamel
Size per group	12
Groups	0 ppm NaF; 550 ppm NaF; 1,100 ppm NaF
Demineralizing solution	calcium – 2.0 mmol/l $Ca(NO_3)_2$ $4H_2O$: 0.47 g/l phosphate – 2.0 mmol/l KH_2PO_4: 0.27 g/l acetic acid – 75 mmol/l CH_3COOH: 4.50 g/l pH 4.4 (adjusted with 50% NaOH)
Remineralizing solution	calcium – 1.5 mmol/l $Ca(NO_3)_2$ $4H_2O$: 0.35 g/l phosphate – 0.9 mmol/l KH_2PO_4: 0.12 g/l KCl – 130 mmol/l KCl: 9.69 g/l PBS – 100 ml/l pH 7.0 (adjusted with concentrated HCl)
Diluent	Deionized water
Treatment	1 min, 3 times/day, for 14 days
Evaluation	QLF; SMH

Table 3. Change from baseline in SMH and mean differences

Groups	Baseline KHN	Final KHN	ΔHV	% change
0 ppm F	93.9±8	107.6±8.8	13.6±1.9	14.9±2.1*
550 ppm F	92.5±7.5	138.8±7.56	46.4±5.6	56.6±9.6*
1,100 ppm F	92.1±6	181.7±10.6	89.6±9.2	103.9±14.6*

Data are described as means ± standard errors. ΔHV = SMH change. * Statistically significant difference compared with baseline ($p = 0.001$).

Results

Surface Microhardness

There were no statistically significant differences in the baseline values of the 3 groups ($p = 0.98$). All groups showed a significant increase in SMH of enamel from baseline to after treatment (table 3). The differences between all pairwise comparisons were statistically significant (group 1 vs. group 2: $p = 0.003$; group 1 vs. group 3: $p = 0.001$; group 2 vs. group 3: $p = 0.001$). The mean percent mineral changes found in the specimens treated with the 3 dentifrices are given in table 3. The placebo treatment resulted in 14.9 ± 2.1% remineralization, the 550-ppm-F dentifrice produced 56.6 ± 9.6% remineralization, and the 1,100-ppm-F dentifrice produced 103.9 ± 9.6% remineralization with respect to the enamel lesion.

Quantitative Light-Induced Fluorescence

The results of the QLF measurements are summarized in table 4. There were no statistically significant differences in the baseline values of the 3 groups ($p = 0.72$).

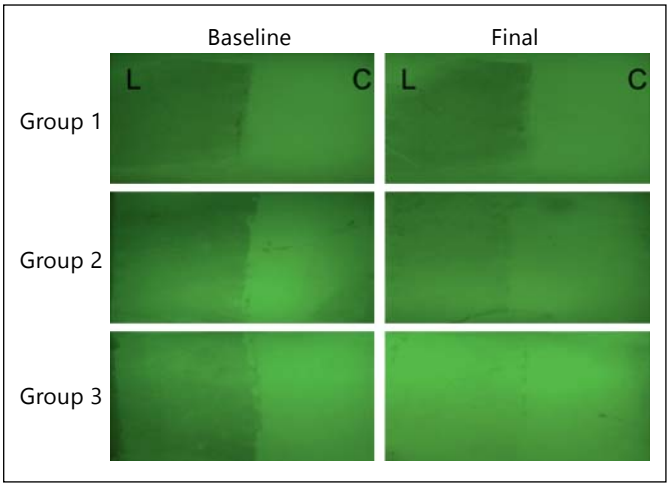

Fig. 2. Examples of QLF images at baseline and after treatment (final). Group 1: 0 ppm F; group 2: 50 ppm F; group 3: 1,100 ppm F. L = Artificial carious lesion area; C = coated area.

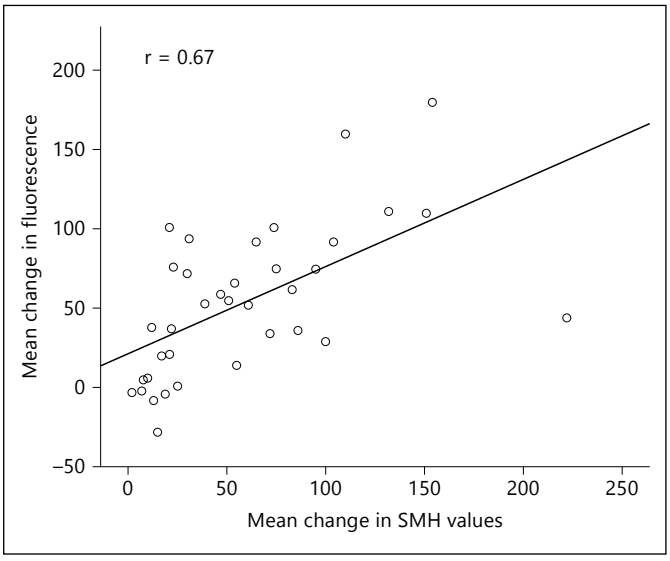

Fig. 3. Plot of SMH values versus mean change in fluorescence.

Table 4. Change in fluorescence values and mean differences

Groups	Baseline	Final	ΔF	% change
0 ppm F	28.6±3.3	25.7±3	2.9±1.7	15.6±7.1*
550 ppm F	20.2±4.4	6.5±4.8	13.8±2.4	59.8±11.9*
1,100 ppm F	24.3±4.5	0.96±4.6	23.2±3.2	85±13.2*

Data are described as means ± standard errors. * Statistically significant difference compared with baseline (p = 0.001). ΔF = Change in fluorescence.

Statistically significant differences were detected between all pairwise comparisons (group 1 vs. group 2: p = 0.002; group 1 vs. group 3: p = 0.001; group 2 vs. group 3: p = 0.006). As expected, specimens in the fluoride groups showed an increase in enamel fluorescence between baseline and after treatment. Figure 2 shows examples of QLF images at baseline and after treatment. The percent fluorescence change resulted in increases of 15.6 ± 7.1% for the 0-ppm-F dentifrice, of 59.8 ± 11.9% for the 550-ppm-F dentifrice, and of 85.1 ± 13.2% for the 1,100-ppm-F dentifrice ($p < 0.05$). The QLF changes were correlated with the SMH changes (r = 0.67; fig. 3).

Discussion

The aim of this study was to compare the ability of QLF and SMH to show differences between toothpastes of different F levels in a remineralization pH-cycling model. Both systems detected statistically significant differences between both fluoride-containing and non-fluoride-containing products within 14 days. The results showed a clear dose response among the dentifrices. To a lesser extent than the F toothpastes, the non-fluoride placebo group also showed mineral gain due to exposure to the remineralizing solution, supersaturated with respect to the enamel.

The results for SMH clearly demonstrate the ability of the two fluoride-containing dentifrices to significantly harden softened enamel relative to the negative control in a pH-cycling model. QLF was also able to detect mineral changes and correlated well with the increase in surface hardness. The results show that the percentage of fluorescence change and surface hardness increased linearly with the concentration of fluoride. The analysis reported in this study compared the change in fluorescence intensity of pre- and postintervention images. Those images can be recorded and repositioned after months, suggesting the possibility of longitudinal monitoring of mineral changes as seen in vivo [Feng et al., 2008].

The current results, while preliminary, suggest that QLF can be used in pH-cycling models to evaluate fluoride effects in artificial caries lesions using human enamel. QLF is based on the principle that demineralized enamel scatters both the light entering the lesion and the fluorescence emitted from the dentine. Hence, the lesion is observed as a dark spot [Amaechi and Higham, 2002]. QLF will detect any mineral loss but cannot make a differential diagnosis; in such cases a

clinical assessment is crucial. The presence of a dentine layer influences light scattering and the absorption properties of the tooth [van der Veen and de Josselin de Jong, 2000]. Therefore, in this study, the samples were prepared including a dentine layer beneath the enamel. The demineralization period (20 min) used in this study was designed to simulate the decreased pH occurring after meals. Fluoride treatments were given before and after demineralization periods. The pH-cycling models attempt to simulate the dynamic conditions that occur in the oral cavity over an extended period of time [Buzalaf et al., 2010]. QLF has the advantage of being a non-destructive method that allows longitudinal analysis of tissues in vitro, in situ or in vivo [Higham et al., 2005]. QLF has also shown the ability to detect and quantify changes in mineral content and size of lesions by demonstrating a dose response between F and non-F dentifrices in short-term clinical trials [Tranaeus et al., 2001; Feng et al., 2007].

In conclusion, the model employed in this study has demonstrated that a system based on fluorescence loss of enamel can detect remineralization as part of a pH-cycling model comparing different concentrations of fluoride. In future investigations, it might be valuable/informative to compare different fluoride concentrations in a demineralization pH-cycling model.

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J.G. performed the experiment, contributed to the manuscript and analysed the data; I.A.P. and R.P.E. contributed to the manuscript and experimental design; R.P.S., B.C., A.R. and I.P. made contributions to the experimental design.

Disclosure Statement

R.P.E., R.P.S., A.R., I.P. and B.C. are employees of the Colgate-Palmolive Company.

References

Al-Khateeb S, Forsberg CM, de Josselin de Jong E, Angmar-Månsson B: A longitudinal laser fluorescence study of white spot lesions in orthodontic patients. Am J Orthod Dentofacial Orthop 1998;113:595–602.

Amaechi BT, Higham SM: Quantitative light-induced fluorescence: a potential tool for general dental assessment. J Biomed Opt 2002;7:7–13.

Bratthall D, Hänsel-Petersson G, Sundberg H: Reasons for the caries decline: what do the experts believe? Eur J Oral Sci 1996;104(pt 2):416–422, discussion 423–425, 430–432.

Buzalaf MA, Hannas AR, Magalhães AC, Rios D, Honório HM, Delbem AC: pH-cycling models for in vitro evaluation of the efficacy of fluoridated dentifrices for caries control: strengths and limitations. J Appl Oral Sci 2010;18:316–334.

Ellwood RP, Goma J, Pretty IA: Caries clinical trial methods for the assessment of oral care products in the 21st century. Adv Dent Res 2012;24:32–35.

Feng Y, Yin W, Hu D, Zhang YP, Ellwood RP, Pretty IA: Assessment of autofluorescence to detect the remineralization capabilities of sodium fluoride, monofluorophosphate and non-fluoride dentifrices: a single-blind cluster randomized trial. Caries Res 2007;41:358–364.

Feng Y, Yin W, Hu DY, Zhang Y, Pretty IA, Ellwood RP: Detection and prevention of early caries after fluoride dentifrice application using quantitative light-induced fluorescence in vivo (in Chinese). Hua Xi Kou Qiang Yi Xue Za Zhi 2008;26:607–610.

Hafström-Björkman U, Sundström F, de Josselin de Jong E, Oliveby A, Angmar-Månsson B: Comparison of laser fluorescence and longitudinal microradiography for quantitative assessment of in vitro enamel caries. Caries Res 1992;26:241–247.

Higham SM, Pretty IA, Edgar WM, Smith PW: The use of in situ models and QLF for the study of coronal caries. J Dent 2005;33:235–241.

Karlsson L: Caries detection methods based on changes in optical properties between healthy and carious tissue. Int J Dent 2010;2010:270729.

Marinho VC, Higgins JP, Logan S, Sheiham A: Topical fluoride (toothpastes, mouthrinses, gels or varnishes) for preventing dental caries in children and adolescents. Cochrane Database Syst Rev 2003;4:CD002782.

Marthaler TM: Changes in the prevalence of dental caries: how much can be attributed to changes in diet? Caries Res 1990;24(suppl 1):3–15, discussion 16–25.

Nyvad B: Diagnosis versus detection of caries. Caries Res 2004;38:192–198.

Pitts NB, Stamm JW: International Consensus Workshop on Caries Clinical Trials (ICW-CCT) – final consensus statements: agreeing where the evidence leads. J Dent Res 2004;83(spec No C):C125–C128.

Pretty IA: Caries detection and diagnosis: novel technologies. J Dent 2006;34:727–739.

Queiroz CS, Hara AT, Paes Leme AF, Cury JA: pH-cycling models to evaluate the effect of low fluoride dentifrice on enamel de- and remineralization. Braz Dent J 2008;19:21–27.

Stookey GK, Featherstone JD, Rapozo-Hilo M, Schemehorn BR, Williams RA, Baker RA, Barker ML, Kaminski MA, McQueen CM, Amburgey JS, Casey K, Faller RV: The Featherstone laboratory pH cycling model: a prospective, multi-site validation exercise. Am J Dent 2011;24:322–328.

Tranaeus S, Al-Khateeb S, Björkman S, Twetman S, Angmar-Månsson B: Application of quantitative light-induced fluorescence to monitor incipient lesions in caries-active children: a comparative study of remineralisation by fluoride varnish and professional cleaning. Eur J Oral Sci 2001;109:71–75.

van der Veen MH, de Josselin de Jong E: Application of quantitative light-induced fluorescence for assessing early caries lesions. Monogr Oral Sci 2000;17:144–162.

White DJ: The application of in vitro models to research on demineralization and remineralization of the teeth. Adv Dent Res 1995;9:175–193, discussion 194–197.